Comm.
Dr. Andervont
Dr. Huebner
Dr. Jacobson

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#935

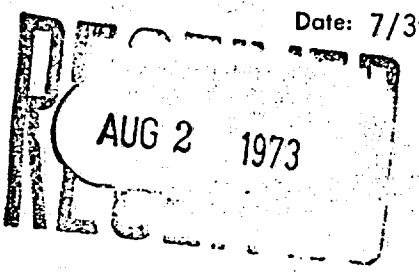
CANCER

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: 7/30/73



1. Principal Investigator (give title and degrees):

William H. Fishman, Ph.D.
Director, Tufts Cancer Research Center
Professor of Pathology
Tufts School of Medicine

2. Institution & address:

Tufts Cancer Research Center
Tufts University School of Medicine
136 Harrison Avenue
Boston, Massachusetts 02111

3. Department(s) where research will be done or collaboration provided:

Department of Pathology
Department of Medicine

4. Short title of study:

Embryonic gene activation in bronchogenic cancer

5. Proposed starting date: October 15, 1973

6. Estimated time to complete: three years

7. Brief description of specific research aims:

The specific research aims are directed toward obtaining at least partial answers to the following questions:

a. To what extent is bronchogenic carcinoma associated with the activation of embryonic genes with respect to four typical carcinoembryonic proteins?

b. Does the individual histologic type of bronchogenic cancer correlate with one or a combination of carcinoembryonic proteins - in the primary lesion? in the metastases?

c. Do carcinoembryonic proteins appear in the bronchial epithelium of individuals destined to develop bronchogenic carcinoma as studied in metaplastic and in situ carcinoma tissue?

d. Is there a correlation between the smoking and drinking histories of patients and the activation of embryonic genes?

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Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

8. Brief statement of working hypothesis:

We believe that a fundamental manifestation of neoplasia is the activation of embryonic, placental and fetal genes while the genes characteristic of adult tissue may continue to be expressed or to a certain extent, suppressed. It is conceivable that the carcinoembryonic genes may, in certain combinations, be responsible for the cell acquiring its autonomous character. For example, placental trophic hormones such as human chorionic gonadotopins, placental lactogen, and thyrotropic hormones, are products of and may have stimulatory effects in a variety of cancers. Conceivably, therefore, although some of the gene products cannot be seen to have any particular relevance to the autonomy of the cancer cell, others do. It is imagined that amongst the first events in a cell undergoing neoplastic change should be the activation of several embryonic genes. On this basis, it may be possible to identify individuals whose ability to undergo activation of embryonic genes in their own cells is greater than it is in other individuals. This would give us some means of evaluating susceptibility or resistance to neoplasia. In the case of cancer of the lung, the present circumstances favor the study of the appearance of embryonic genes in bronchial epithelium of individuals with and without bronchogenic cancer. It is also possible that a knowledge

(contd. on separate sheet)

9. Details of experimental design and procedures (append extra pages as necessary)

Details of experimental design and procedures

The project can be visualized as developing in two stages; first, the "phasing-in" process and second, the initiation and operation of the fully organized study.

"Phasing-in"

We would plan to test the adequacy of our tissue and cell sampling techniques, the completeness of extraction of the various carcinoembryonic proteins and the reproducibility of the radioimmunoassay procedures on specimens of bronchogenic cancer, bronchial epithelium, etc., which we have available in cold storage or which present themselves at surgery.

Similarly, the pathologist would develop and standardize the practices which will be the most optimal in order to ensure selection of representative specimens, the labeling techniques which would be the most foolproof and the establishment of a routine from beginning to end with a concern for objectivity in reporting the histopathologic findings.

Obviously, the scope of the study bears some relation to the autopsies and surgicals to be performed. A measure of this facet is provided by the following table.

PATHOLOGICAL MATERIAL - PONDVILLE HOSPITAL

Site of Cancer	New Patients	1972 Autopsies	1973 Autopsies	1974 Autopsies
Lung	65	26	PROJECTED	PROJECTED
Breast	117	19		
Colon	43	5		
Prostate	22	3		
Bladder	12	3		
Stomach	7	0		
Other	428	43		
	694	99	109	120

(contd. on separate sheet)

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Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

8. Brief statement of working hypothesis (contd.)

of the nature and extent of embryonic gene expression in different types of bronchogenic carcinoma could be valuable in estimating the prognosis in individual patients. Since these embryonic gene products are normally foreign to the host and since they are able to get into the bloodstream, the potential for serodiagnosis by radioimmunologic measurements exists. The resulting information could give the physicians an indication more broadly based than on the H and E section alone as to the "activity" of the bronchogenic cancer and could provide an objective means of monitoring the disease.
We have preliminary evidence that in a population of alcoholics the Regan isoenzyme is expressed in a high percentage of cases. [In this connection, we have been wondering about the significance of an alcoholic patient whose serum was Regan-positive and two years later developed an undifferentiated cancer of the lung.] If individuals who consume alcohol have a predilection to activate embryonic genes, then these individuals may become high risks for bronchogenic, and head and neck cancers if atmospheric irritants represent an additional contributing factor for activating the embryonic genes further.

With the availability of immunohistochemical techniques, it should be possible to identify the individual cells in the normal and malignant bronchial epithelium which are expressing the various embryonic gene products. These would include Regan isoenzyme, CEA, HCG, and α-fetoprotein.

This working hypothesis has developed from our own studies on the Regan isoenzyme (placental-type alkaline phosphatase) in cancer and non-cancer patients (see reprints) and from the experiences of others with CEA and α-fetoprotein. We have found that abnormal amounts of Regan isoenzyme were present in the serum of one out of seven patients and in a number of conditions predisposing to or associated with pre-neoplastic situations (Nathanson, L., and Fishman, W.H., Cancer, 27: 6, 1388-1397, 1971; Stolbach, L.L., Krant, M.J., and Fishman, W.H., N.E.J. Med., 281: 757-762, 1969). With the development of ultrasensitive techniques, we are now finding a greater percentage of cancer patients whose serum is Regan-positive along with an appreciable incidence of trace amounts in individuals who are presumably healthy. This experience fits the results others are reporting for CEA and α-fetoprotein and suggests that embryonic gene activation is taking place in cells of normal individuals at a low rate and that the appearance of higher titers in malignancy represents a quantitative rather than a qualitative manifestation.

The choice of four carcinoembryonic proteins (Regan isoenzyme, HCG, CEA, α-fetoprotein) is not meant to exclude other proteins which are not proven carcinoembryonic as yet but which are now beginning to attract attention. For example, Gewirtz and Yallow at the 55th Annual Meeting of the Endocrine Society reported that big and little forms of ectopic ACTH can be distinguished and that it is the former which is immunoreactive but not biologically active. Amongst other finding, they state that five of five bronchogenic carcinomas and one bronchial adenoma obtained at surgery contained ACTH, while none was detectable in two surgical specimens of normal human lung. Also, in our laboratory, we are examining a varity of placental enzymes and proteins with a view to identifying additional carcinoplacental proteins. As soon as this is successful, we would plan to include measurements of these additional proteins in the lung cancer study.

The relationship of the activation of embryonic genes as compared to activation of oncogenes still remains to be established. It is our hope that success in the work projected will be helpful in the examination of the oncogene hypothesis which is the challenging theory of neoplasia today.

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Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

9. Details of experimental design and procedures (contd.)

In our opinion, there is more than an adequate supply of suitable cases (60% autopsy rate of patients dying at Pondville Hospital) and our habit is to carry out as thorough and complete studies as possible. Also, since Pondville Hospital accommodates cancer patients only, the comparisons initially will be the bronchial epithelium and tumors of lung cancer patients versus the bronchial epithelium of patients with cancer at other sites.

Tumor tissue will be processed for phenotypes in the case of the non-lung cancer patients whose bronchial epithelium is included iin the study.

HISTOLOGIC CLASSIFICATION OF LUNG CANCER

1. Squamous cell carcinoma, well, moderately or poorly differantiated

2. Small cell carcinoma (including oat cell)

3. Adenocarcinoma (including bronchiolar), well, moderately, poorly differentiated

4. Large cell anaplastic carcinoma (undifferentiated carcinoma)

5. Mixed tumors will be classified according to the predominant cell element and other types will be stated

Pathologic Study

It will be necessary to establish the number of samples to be taken from a given area of bronchial epithelium to give reproducible results. In the event abnormal epithelium is visible, cell suspensions of abnormal epithelium will be made separately from those of normal-appearing epithelium. In addition, some blocks of the trachea will be submitted to routine hematoxylin and eosin staining for a point of reference.

At autopsy we will obtain the distal 3 cms of trachea, right and left main stem bronchi to the point of bifurcation. Tissue will be taken for histology from (a) the proximal end of trachea, (b) the carina, (c) at the bifurcation of main stem bronchi.

This will be standard procedure in control cases as well as carcinoma of lung, presuming the pathologic process has not infiltrated bronchi.
If there are pathologic lesions involving the above areas, we would get as much normal mucosa as possible, 2-3 cms from the lesions from either bronchi or trachea.
In cases of carcinoma of lung, we will collect tissue where it appears most viable, preferably in the following order:

a. primary site
b. regional lymph nodes
c. metastases to distant organs

A minimum of two sections per block will be processed histologically for this particular project with blocks and slides, which will be filed separately.

Collection of bronchial epithelial cells

Several alternatives are under consideration.

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Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

9. Details of experimental design and procedures (contd.)

One possibility is to dissect out the mucosal epithelium and to check the completeness of its removal by histologic examination of the remaining tissue. Another approach is to use the technique of Dr. B. Spencer, published in the Journal of Histochemistry and Cytochemistry, 6: 105, 1958. In this technique, autopsied specimens of bronchus and trachea are trimmed of lymphatic and fatty tissue and cut into pieces about 2 cm square. Superficial mucous is removed by lightly brushing the surface of the epithelium with a No. 5 camel hair brush in the presence of the liquid medium at 0°. A portion of the epithelium is then isolated by pressing onto it a lucite tube of 1 sq. cm cross sectional lumen area so as to form a water-tight seal. Liquid medium (0.2 ml) is added into the tube and the epithelium brought into suspension using a No. 2 lettering brush with the bristles trimmed to a length of 4 mm. The most satisfactory results are obtained by twirling the brush between the thumb and forefinger and maintaining a very light downward pressure. The suspension is removed with a transfer pipette and the process repeated with two further 0.2 ml portions of the liquid medium and the suspensions combined. The epithelial suspension so prepared consists of clumps of cells which are broken up by repeated passage through a pipette with an orifice of 0.1 - 0.2 mm. A portion of the suspension is retained for cell counting and the remainder distributed among a number of small tubes which are capped with parafilm and stored at -20° until required for biochemical assay. With this technique, normal epithelia of both bronchus and trachea possess between 2 and 3 million cells per sq. cm and a ratio, intermediate:goblet:ciliated:basal cells appears to be of te order of 0.2:0.4:1.0:1.6 and the ratio, columnar:basal cells is approximately 1:1. The chief value of this technique is that it provides a means of relating the chemical properties of epithelium to the cytological composition of th tissue since assays for carcinoembryonic proteins and differential and total cell counts are made on the same suspension.

Determination of carcinoembryonic proteins

Once it has been established how the tissue specimens and cell suspensions can be aliquoted and best preserved for the subsequent assays and best processed to release all embryonic protein into solution, the following techniques will be employed.

(a) the proximal end of trachea, (b) the carina, (c) at the bifurcation of main

Regan Isoenzyme. This alkaline phosphatase will be extracted and assayed according to the procedures used originally to accomplish this purpose (Fishman, W.H., Inglis, N.R., Stolbach, L.L. and Krant, M.J., Cancer Res., 28: 150-154, 1968). The confirmation of Regan isoenzyme as a placental alkaline phosphatase will be made by Ouchterlony double-diffusion techniques (Fishman, W.H., Inglis, N.R. and Green, S., Cancer Res., 31: 1054-1057, 1971) and by microzone membrane electrophoretic technique (Inglis, N.R., Guzek, D.T., Kirley, S., Green, S. and Fishman, W.H., Clin Chim Acta, 33: 287-292, 1971). The detection of the Nagao type of isoenzyme will be made on the basis of L-leucine inhibition (Inglis, N.R., Kirley, S., Stolbach, L.L. and Fishman, W.H., Cancer Res., 33: 1657, 1973). The results will be expressed as indicated in Placental isoenzyme units using an appropriate term such as, per gram of tissue or per million cells or per mg DNA.

HCG - Human Chorionic Gonafotropin. We intend to use the radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone (Vaitukaitis, J.L., Braunstein, G.D. and Ross, G.T., Am. J. Obstet. Gynecol., 113: 751-758, 1972). In this assay, tracer quantities of the beta subunit of human chorionic gonadotropin (HCG-β) is iodinated with ^{125}I by the chloramine-T method to a specific activity of 50 to 100μCi/ug. The reference preparation is a highly purified HCG preparation. Two hundred microliters of serum or plasma from patients is assayed and the same amount of outdated male

10035439 46

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9. Details of experimental design and procedures (contd.)

blood bank plasma is added to all background and standard tubes to obviate a non-specific plasma protein effect. The tubes are incubated for 2 hours at 37°C and then for an additional 15 to 17 hours at 4°C. Then the sheep anti-rabbit serum is added and the incubation is continued for an additional 6 to 12 hours at 4°C. Separation of bound and free hormones by centrifugation is followed by measurement of the bound radioactive trace in a gamma counter. The within assay coefficient of variation is 15% and the between assay coefficient of variation is 27% for four replicate samples that range from 7.3 to 19.7 mg/ml.

CEA - Carcinoembryonic Antigen of Gold. The original radioimmunoaasay method of Thomson, D.M.P., Krupey, J., Freedman, S.O. and Gold, P., Proc. Natl. Acad. Sci., 64: 161-167, 1969, requires 5 ml of serum which is extracted with perchloric acid. Following centrifugation, dialysis, lyophilization, resuspension in antibody-containing solution, the mixture is incubated with labeled antigen and the bound labeled antigen separated by centrifugation of its ammonium sulfate solution. We are attracted to the recently published radioimmunoelectrophoretic binding assay of Coller, J.A., Crichlow, R.W., and Yin, Lo Ke, Cancer Res., 33: 1684-1688, 1973, which involves simultaneous electrophoretic binding and separation with the use of 10µl of whole sera and requires only two hours.

α-Fetoprotein. Radial immunodiffusion techniques such as those of Abelev (Cancer Res., 28: 1344-1350, 1968) and of Alpert, M.E., Uriel, J. and DeNechaud, B. (New Eng. J. Med, 278: 984-986, 1968) are standard methods for measuring α-fetoprotein in serum.

More sensitive and rapid techniques are now available. The one which appeals to us in the electroimmunodiffusion method of Sizaret, P.P., McIntire, K.R., and Prinder, G.L. (Cancer Res., 31: 1899-1902, 1971) in which a linear relationship is established between antigen concentration and length of precipitin (Ag-Ab) peak. Especially when the antiserum is radioiodine-labeled, the sensitivity of the test is greatly increased.

Clinical Study

Each patient will receive a physical examination on admission and a blood specimen would be collected for the assay of carcinoembryonic proteins. Questionnaires (examples attached) will be filled out by the patient regarding the history of smoking and of drinking. Abstract of pre-admission medical history and a synopsis of the patient's history while in the hospital will be prepared separately for each patient. Since one cannot know in advance which patients will come to autopsy, it will be necessary to evaluate every admission.

Information from smoking and drinking histories plus the histologic diagnosis of the tumor plus the laboratory data on carcinoembryonic proteins should provide sufficient data on which to base a decision whether or not to expand the study to other populations of patients. These non-cancer subjects are readily available at the Shattuck and New England Medical Center Hospitals, institutions which are an integral part of the operation of Tufts Cancer Research Center.

Finally, the team would meet at regular intervals to evaluate their individual roles, to work out problems in coordination of effort and to decide on the best way to collect and analyze the information from both qualitative and statistical points of view.

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9. Details of experimental design and procedures (cont.)

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Our facilities at Tufts University School of Medicine occupy the entire 4th floor of the South Cove building and include a research area of over 4,000 square feet. Tufts University provided the financial resources for the complete renovation and construction of the Cancer Research laboratory. In addition, the Massachusetts Division of the American Cancer Society made a grant to equip the laboratory with several of the most modern pieces of essential equipment. A brief description of the new facilities and equipment follows.

We have a modern cold room laboratory equipped with air, vacuum, electricity and water. In addition, there is sufficient space to house two large fraction collectors equipped with two UV monitors with two recorders. A separate service room is available which houses a Heinicke dishwasher completely overhauled and updated, well insulated steam drying ovens, a small autoclave as well as a large sink for manual glassware washing. There is also sufficient shelf space to store all essential items needed for the routine housekeeping of the laboratory. A solvent storage closet has also been provided. One major facility is the electron microscopy room separate from the EM power supply room, and an adjacent dark room equipped with a 7' photography sink and a Simmons Omega enlarger capable of producing 8" by 10" enlargements. The EM room houses a new JEOLCO 100B transmission electron microscope. The entire suite is isolated from the main laboratory and is designed to maintain proper constant temperature and humidity at all times. In addition, there is an electron microscopy preparation room with an LKB ultramicrotome. Our facilities also include a tissue culture room complete with an Edgeguard laminar flow hood and a large National tissue culture incubator. This room has constant temperature humidity, absolute air filtration and a germicidal light. The instru-

11. Additional facilities required:

None.

the test is greatly increased

each patient will receive a physical examination on admission and a blood specimen would be collected for the assay of carcinoembryonic protein. Questionnaires

information from smoking and drinking histories plus the histo

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

10035439 48

Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

10. Space and facilities available (contd.)

ment room also maintains constant humidity and houses the Beckman DKI, DU, the Coleman 44, Mettler balances, Cahn micro electric balance, plus other items. This instrument room houses previously purchased equipment at the present time. The special photography room is equipped with a Polaroid MP-3 camera plus an air incubator and a Chromatovue Fluorescent viewing cabinet, all of which are employed to develop, observe and photograph the different alkaline phosphatase isoenzymes either by employing cellulose acetate membrane electrophoresis with their detection subsequently with a fluorogenic substrate or by using starch gel electrophoresis with a chromogenic-dye coupler substrate.

The main research laboratory area occupies approximately 3,000 square feet and it is fully air conditioned. The laboratory is sub-divided into areas specifically designed to perform automated enzymology and other related methods, manual enzymology, all types of electrophoresis, protein purification, immunology, histochemistry and light microscopy and radioisotope studies. In addition, we have the use of the Medical School's animal farm which is easily accessible to us for housing our experimental animals. The general laboratory has a 7' radioisotope hood and a 4' fume hood. All laboratory benches have small undercounter refrigerators and freezers for short term use. We have also acquired a Spinco L-2 65B ultracentrifuge, two Sorvall RC-2 centrifuges and a Sorvall GLC-1 centrifuge. These items, in addition to the Nuclear Mark I isotope counter and other centrifuges, are housed in the general laboratory. A separate open storage area distinct from the general laboratory houses the new Kelvinator -76° freezer used for antigen and antiserum storage, the Scotsman ice machine, the Virtis lyophilizer and shell freezer, in addition to several other large freezers and refrigerators.

Finally, communication between our staff is maintained by means of a conference room equipped for oral, written and slide presentations. Adjacent to the conference room is the Director's office. A secretarial office and an office for Sidney Green, Senior Chemist and laboratory manager, completes the administrative suite.

10035439 49

Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

14. First year budget:

A. Salaries (give names or state "to be recruited")		% time	Amount
Professional (give % time of investigator(s) even if no salary requested)			
W. H. Fishman, Ph.D.	Responsible Investigator	25	*
L. L. Stolbach, M.D.	Co-Investigator	10	3,500
L. Gandbhir, M.D.	Co-Investigator	15	5,000
C. H. Chang, Ph.D.	Co-Investigator	100	15,000
S. Raam, Ph.D.	Research Fellow	30	3,000
H. Miyayama, M.D.	Research Fellow	25	*
G. Doellgast, Ph.D.	Research Fellow	25	*
Technical			
M. L. Orcutt, M.S.	TCRC Lab Res. Asst.	100	9,000
to be recruited	Pondville Res. Asst.	100	8,000
Fringe benefits on all salaries			5,655

*Institutional Funds

Sub-Total for A: 49,155

B. Consumable supplies (by major categories)

Plastic ware, reagents for radioimmunoassay, reagents for tissue purification, glassware, histochemical and histological supplies

okay gk

Sub-Total for B: 5,000

C. Other expenses (itemize)

Travel between Pondville and the Cancer Research Center in connection with pickup of biological materials

Sub-Total for C: 500

Running Total of A + B + C: 54,655

D. Permanent equipment (itemize)

None.

Sub-Total for D: 0

E. Indirect costs (15% of A+B+C): 8,198

Total request: 62,853

15. Estimated future requirements: Res. Asst. at Pondville starting 02 year at 9,040; 7% annual salary increases

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	61,636	6,000	600	0	10,235	78,471
Year 3	65,951	6,000	600	0	10,883	83,434

10035439 50

Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Career Award	National Cancer Institute 4K06CA18453-11	29,540	7/1/73 - 6/30/74
2. Fundamental Enzymologic Studies Applied to Oncology	NCI - 7R01CA13332-02	84,020	1/1/73 - 12/31/73
3. Tufts Cancer Research Center	NCI - CA 12924-02	8,727	12/1/72 - 11/30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Fundamental Enzymologic Studies Applied to Oncology	NCI - 7R01CA13332-03	79,506	1/1/74 - 12/31/74
2. Renewals of other above grants are planned			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator

Typed Name William H. Fishman, Ph.D.

Signature William H. Fishman Date 7/30/73

Telephone 617 (Area Code) 423-4600 (Number) 523 (Extension)

Checks payable to

Tufts University School of Medicine

Mailing address for checks

136 Harrison Avenue

Boston, Massachusetts 02111

Responsible officer of institution

Typed Name Joseph J. Byrne, Ph.D.

Title Research Coordinator

Signature Joseph J. Byrne Date 7/30/73

Telephone 617 (Area Code) 423-4600 (Number) 428 (Extension)

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Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

W. H. Fishman, Ph.D.
Tufts Cancer Research

APPENDIX

Question

1. Background information
   a. Personal
   b. Tufts Cancer Research Center
   c. Cancer Cell Phenotype Program
   d. Scientific Staff (new)
2. Institutional Commitment
   a. Physical Plant
   b. Faculty Tenure
   c. Operating Expense
3. Departmental Resources
   a. Pathology
   b. Medicine
   c. Pondville Hospital
4. Relationship to Direction of Total Research Effort of TCRC

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Source: https://www.industrydocuments.ucsf.edu/docs/gbmm0050

1. Principal Investigator - Background

a. Personal

The principal investigator has completed 25 years as a Professor of Tufts University School of Medicine, during which time he has been identified with cancer research and cancer teaching. In 1960, his professional competence was recognized amongst other awards by a lifetime NCI Career Professorship Award. He has maintained a balance between fundamental research and clinical application and has been successful in developing and holding together a team of Research Assistants and Faculty collaborators. It was this Cancer Research Laboratory which discovered the Regan isoenzyme (a placental alkaline phosphatase) in a patient with cancer of the lung and had earlier devised techniques for fractionating serum alkaline and acid phosphatases according to their principal organ source. A continuing interest in acid hydrolases (β-glucuronidase, and acid phosphatase) in human tumors and in hormone induction of enzyme activity is evident. As editor, he has just published through Academic Press, Volume 3 of "Metabolic Conjugation and Metabolic Hydrolysis."

With regard to his major research commitment, he has been invited to help organize the "International Research Group for Carcinoembryonic Proteins" at a Symposium to be held in Sapporo, Japan, October 22-25, 1973.

b. Tufts Cancer Research Center - TCRC

This was established January 1, 1972 by virtue of an award by the National Cancer Institute to establish a multidisciplinary Cancer Research Center at Tufts University School of Medicine. The scientific mission of this Center is to expand knowledge in the area of Cancer Cell Phenotypes, and to identify and utilize those of real clinical utility. This mission is what makes the TCRC unique.

As a new entity at Tufts University School of Medicine, the Cancer Research Center does have a position of leadership within the Tufts - New England Medical Center Complex because of its entirely interdepartmental program. It has the support of the departmental Chairmen who have helped to integrate it into the organization of the Medical School, the New England Medical Center Hospital and the other Tufts hospitals. Especially relevant is the wide participation of the Faculty in cancer teaching. Next, leadership within the Cancer Research Center is centered on the Director, who is given the responsibility for the management of the Center and the authority necessary for the effective performance of his duties. He, in turn, operates as a member of the Executive Committee which establishes the overall institutional policies of the Center.

In the area of clinical research and cancer training, three Tufts hospitals are already working closely together as members of the Eastern Cooperative Oncology Group. They are the New England Medical Center Hospital, Pondville Hospital and Lemuel Shattuck Hospital.

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Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

1. b. (contd.)

In addition, a CRC bed unit at the New England Medical Center Hospital is to be the focal point for the application of research findings in cancer biology to clinical cancer therapy.

Aside from the role of the Center at Tufts itself, its scope extends to many parts of Boston and to three New England states. Thus, the Boston area is covered by NEMCH and six other Tufts hospitals; Massachusetts, New Hampshire and Rhode Island are included in the tri-state Regional Medical Program, with which Tufts is associated.

For further details, please consult reprint entitled "Tufts Cancer Research Center Opened and Dedicated May 24, 1972".

c. Cancer Cell Phenotype Program

By cancer cell phenotypes, we refer particularly to those which are of greatest interest and relevance to cancer as will be described in the following.

Clinical symptoms or laboratory findings in a cancer patient can often be explained as resulting from the expression of traits or phenotypes of the cancer cell. These phenotypes can be classified into two groups: those which are the gene product similar to that of cells from which the tumor originated (expected) and those which are the gene product different from that of cells from which the tumor originated (unexpected or "ectopic").

Examples of the "expected" category are the production of γ-globulin in multiple myeloma, of insulin in islet cell cancer of the pancreas, of adrenocorticoid hormones in adrenal carcinoma, of gonadotrophin in choriocarcinoma, etc. In the "ectopic" category, one usually points to the production of ACTH, calcitonin, parathormone, gonadotrophin, lactogenic hormones, or anti-diuretic hormone in cases of cancer of the lung, of α-1-fetoglobulin in hepatomas and teratomas, of carcinoembryonic antigen in cancer of the gastrointestinal tract, of Regan isoenzyme in many types of cancer and of muscle aldolase in hepatoma. A special category of phenotypes is the viral tumor antigens.

These findings have proven to be extremely significant in solving problems of the differential diagnosis of cancer and in objectively evaluating the effects of therapy. For example, gonadotrophin production in a non-pregnant female with evidence of tumor points to the possibility of choriocarcinoma, for which specific curative therapy is available. The presence of elevated values for "prostatic" acid phosphatase in a male with urinary symptoms points to a strong possibility of disseminated cancer of the prostate for which specific palliative therapy is indicated. A good therapeutic result is predicted by a rapid fall of the "prostatic" acid phosphatase and recurrence usually by a return of elevated values.

In the case of all of the "expected" phenotype category, the recognition of the tumor product directs the physician's attention to the probable organ site of the tumor.

The measurement of various antigenic materials produced by tumors offers considerable potential for diagnosis as well as serving as an indicator of progression or regression of tumor.

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Source: https://www.industrydocuments.ucsf.edu/docs/gtcm0000

1. c. (contd.)

The present techniques differ in many ways depending on which polypeptide hormone or carcinoembryonic antigen is being measured. So far, no attempt has been published of the simultaneous determination of several of these phenotypes but rather, the studies deal with single phenotypic proteins. All of these techniques are handicapped by the requirement of large quantities of serum and by long, tedious procedures. The desirability of establishing a cancer cell phenotype profile for individual cancer patients is clear with the use of improved analytical techniques.

At the present time these phenotypes can be classified as follows into several categories.

A. Carcinoembryonic Proteins

- carcinofetal proteins
  - CEA
  - fetoproteins
  - heterophile fetal antigen
  - leukemia-associated antigen
  - fetal sulfoglycoprotein antigen
  - fetal isoenzymes
- carcinoplacental proteins
- placental alkaline phosphatases
  - Regan isoenzyne
  - Nagao isoenzyme
- placental hormones
  - chorionic gonadotrophin
  - placental lactogen
  - plasminogen activators
- tumor angiogenesis factor

B. Non-carcinoembryonic Proteins

- viral antigens
- cell surface antigens
  - TSTA
- non-placental polypeptide hormones
  - ACTH
  - ADH
  - TSH
  - calcitonin
- host proteins in general

Non-carcinoembryonic proteins are being studied in other goals of the phenotype program, e.g., cell surface antigens in Tumor Immunology, TSTA and viral antigens in Viral Oncology, and calcitonin of medullary thyroid carcinoma in Diagnosis and Therapy.

Ovarian Cancer

Ovarian carcinoma appears to be suitable as a prototype for a systematic phenotype study. In a high percentage of cases after surgery, recurrence is manifested by multiple peritoneal implants which produce ascites. The clinical necessity of relieving this ascites results in the collection of ascitic fluid often in volumes of several liters. This fluid usually contains cancer cells, mesothelial cells, lymphocytes, etc. By cloning such mixtures of cells in tissue

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culture, it should be possible to grow out cancer cells which would be recognizable by a distinctive phenotype. In this regard, ovarian cancer exhibits a high incidence of Regan isoenzyme which provides a highly useful "marker".

This ascites fluid is a resource for cancer-related phenotypes which have not yet been characterized and also provides an indication of the effects of therapy on cancer cells by the measurement of known phenotypes. Thus, in Dr. Fishman's laboratory, the ovarian cancer ascites fluid has been a rich source of the Nagao isoenzyme (a variant of Regan isoenzyme which is L-leucine-sensitive), of transferrins (which Dr. Drysdale is studying) and of histaminase (which is being investigated by Dr. C. W. Lin). Also, Dr. L. L. Stolbach has observed alterations in phenotype during the course of therapy of his patients. The cancer cells have revealed interesting features of mitochondrial membrane alkaline phosphatase when examined by electron microscopy (Dr. M. Sasaki). The organization of this study is the responsibility of W. H. Fishman and L. L. Stolbach.

Other general goals of the Cancer Cell Phenotype Program are defined as "Tumor immunology and cancer-specific antigens" (R. S. Schwartz, L. Nathanson, M. Flax, S. Leskowitz are several of the thirteen investigators involved); "To develop new knowledge on the mechanism of gene expression" (G. Brawerman, C. Sonnenschein and others); and "Viral oncology" (S. Tevethia, R.S. Schwartz, G. Wright).

d. Scientific Staff

The senior staff of Center investigators includes an immunoviral oncologist and a cancer cell biologist.

Coming from Baylor University, Dr. S.S. Tevethia, our immunologist, has joined the staff as of July 1, 1973 as an Associate Professor of Pathology. He is a recognized source of strength in the study of cell surface antigen phenotypes in cells transformed by viruses. Thus, cells transformed by oncogenic viruses acquire tumor-specific transplantation antigen (TSTA) which mediate the development of the cellular immune response of the host leading to the rejection of tumor cells carrying the same antigen. Although immune response to TSTA has been studied in detail, few attempts have been made to understand the basic nature of this antigen or its genetic origin. Tevethia's overall objective is to study the appearance of TSTA during the viral reproductive cycle and the viral abortive cycle and to determine its genetic origin with the use of temperature-sensitive mutants.

Dr. Carlos Sonnenschein, our cancer cell biologist, is a Career Development Awardee of the National Cancer Institute, who has strong interests in cell genetics, including somatic cell hybridization, and in the elaboration by the cell of "estrogen receptor" proteins. These "estrogen receptor" proteins represent phenotypes of a transformed rat uterine endometrial cell line that Sonnenschein has developed.

Both Drs. Tevethia and Sonnenschein's laboratories will be located, as of January 1, 1974, in the TCRC laboratories in the South Cove Building, which will occupy four floors when completed. Both will be alert for opportunities for investigation which the current research proposal might open to them in the future.

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Other Tufts scientists who are working in close cooperation with the Director in developing the Cancer Cell Phenotype program are listed below:

Robert S. Schwartz, Professor of Medicine: Immunologic activation of leukemic virus
Larry L. Nathanson, Associate Professor of Medicine: Melanoma, clinical bed unit
Seymour Reichlin, Professor of Medicine: Medullary thyroid carcinoma
Leo L. Stolbach, Associate Professor of Medicine: Chemotherapy
S. Leskowitz, Professor of Pathology: Immunochemistry
G. Brawerman, Professor of Biochemistry: Molecular biology
H. Drysdale, Associate Professor of Biochemistry: Fetoproteins

Staff Recruited for TRC Project

With regard to the specific objectives of grant application, two individuals have been recruited who are not only well qualified, but were attracted by the cancer cell phenotype program over others which had been offered to them. Regarding Dr. C-H. Chang (see attached curriculum vitae), Dr. Florence Moog, Professor of Biology in Washington University, who supervised his Ph.D., writes "He proved to have all the prerequisites of a productive research scientist: imagination, technical versatility, resourcefulness, persistence and boundless energy"... "One thing I can assure you of, however, is that he is exceptionally talented in mastering new techniques, quickly and with great thoroughness". Dr. Sidney Udenfriend, Director of the Roche Institute of Molecular Biology, states "Dr. Chang is a very capable experimental scientist and is highly motivated. This has been a very profitable year for him because he has mastered many new techniques, including radioimmunoassay, tissue culture, isoelectric focussing, affinity chromatography and immunology".
Dr. Chang is joining us as Assistant Professor of Pathology and is expected to assume an increasing important role in the direction and execution of this research program under your consideration.

Dr. Shanthi Raam has just received her Ph.D. in immunology from Dr. F.P. Inman at the University of Georgia. He writes "She is familiar with short-term cultures of murine tumor cells, radiolabeling (^{3}H) tumor cells proteins, preparation of intracellular fluids, density gradient centrifugation, analytical polyacrylamide gel electrophoresis, preparative block acrylamide gel electrophoresis, liquid scintillation counting and preparation of specific antisera. Dr. Raam is joining us as a post-doctoral research fellow on August 15th and will be working with Dr. Chang and Dr. Fishman to validate the radioimmunoassay techniques for the various carcinoembryonic proteins mentioned in the application.
Two pathologists will be engaged in this research project.

Dr. Lalita Gandbhir is Chief of the Pathology Service at the Pondville Hospital, a Board Member in Anatomic and Clinical Pathology who received much of her training at University Hospital, Boston University School of Medicine. She also engaged in research projects of the Mason Research Institute in toxicologic pathology. She was appointed to Pondville at the same time as Dr. L. L. Stolbach was made Chief of Medicine. She has been collaborating actively

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with him in protocol studies of the Eastern Cooperative Oncology Group and is enthusiastic about the possibility of engaging more deeply in fundamental cancer research based on pathologic material. She has cooperated well with Drs. Stolbach and Fishman in securing tissue specimens from patients with ovarian cancer and other malignancies.

Dr. H. Miyayama is a professionally qualified pathologist who has been trained in the techniques of enzyme histochemistry and of electron microscopy in Professor T. Takeuchi's laboratory in Japan. Professor Takeuchi has had a long professional relationship with Dr. Fishman and sends him his best young pathologists. Dr. Miyayama is located in the TCRC laboratories and would participate with Dr.Gandbhir in working out the best sampling, histologic and cytologic approaches to the evaluation of carcinoembryonic proteins in cells.

Other Faculty who will participate in the project are referred to under Departmental Resources.

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2. Institutional Commitment

a. Physical Plant - Commitments

The University has purchased the South Cove Building which is to house the Tufts Cancer Research Center on half of its eight floors. The first completed floor housing Cancer Enzymology and the Office of Director represents an additional Tufts investment of $120,000. The second, third and fourth floors will require the investment of at least $500,000 of "hard" University funds of which a third has been pledged. Other commitments for support have come from the National Cancer Institute, which has awarded $800,000 for alterations and renovations in 1972 and has approved $150,000 in addition for 1973.

b. Faculty Tenure

At the time of the original TCRC application, Tufts had committed itself to underwriting four tenured professorships, two of which are now in existence (Drs. Fishman and Tevethia). The remaining Professorships will be phased in as expansion of laboratory facilities takes place.

c. Operating Expense

Here the Institutional annual contribution to salaries amounts to $53,000 and to the operation of the present laboratories, $18,000. With the completion of additional two floors in 1973, the operating expense would triple ($54,000).

3. Departmental Resources

a. Pathology

As a Professor of Pathology, Dr. Fishman can obtain, as necessary, the help of his colleagues such as Dr. M. Flax, Chairman of the Pathology Department, and Dr. H. Wolf, Chief of Pathology at the New England Center Hospitals. Both of these men were trained at the Massachusetts General Hospital. These resources are also available to Dr. Gandbhir, the Pondville pathologist who received her training at Boston University Hospital, with Drs. Ira Gore and Max Goodman. In addition, working in Dr. Fishman's laboratory as head of the enzyme histochemistry section is Dr. H. Miyayama, who is a pathologist with excellent training from Dr. Takeuchi's laboratory. He would be working in close cooperation with Dr. Gandbhir in the handling and study of the specimens of bronchial epithelium and bronchogenic carcinoma.

The enzyme histochemical expertise in Dr. Fishman's laboratory is directed towards the fashioning of specific immunochemical techniques using the horseradish peroxidase label along the lines developed by P. Nakane and H. Sternberger for the recognition of polypeptide hormone producing cells of the pituitary gland. Once this capability is achieved, we would be in a position to attempt to demonstrate the various placental and fetal proteins in the cells of the neoplastic and pre-cancerous bronchial epithelium.

b. Medicine

The Medical Oncology component of the Department of Medicine at Tufts is an integral part of the Tufts Cancer Research Center and Dr. Nathanson is to be in charge of an eight bed clinical unit at the New England Center Hospital in addi-

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tion to operating an active oncology service. Dr. Leo L. Stolbach is Chief of the Medical Service at Pondville Hospital which is an all-cancer state hospital. Dr. J. L. Cohen is Chief of the Oncology Service at the Lemuel Shattuck Hospital. These three clinical investigators are active in the Eastern Cooperative Oncology Group studies evaluating chemotherapeutic regimes for breast cancer, lung cancer, melanoma, ovarian cancer, etc. Dr. Stolbach has been associated with Dr. Fishman since 1956, when he worked in the laboratory as a medical student. At different stages in the development of his career, amongst other pursuits, he collaborated with Dr. Fishman in the study of the isoenzymes of alkaline phosphatase and participated in the discovery of the Regan isoenzyme and in its clinical evaluation. Dr. Stolbach received his training in endocrinology from Dr. Roy Hertz at the National Cancer Institute. He has very good rapport with Dr. Gandbhir and with the administration of the Pondville Hospital.

c. Pondville Hospital

The Pondville Hospital is a 110 bed State hospital in Norfolk (Walpole), Massachusetts, which is devoted entirely to the care of patients with cancer and related diseases. The average occupancy during the past few years has been approximately 76 patients. A modern new hospital complex was completed in 1972. The plans include the most up to date radiotherapy equipment including a betatron and a modern automated clinical laboratory. The present buildings will be turned over to other uses, including conversion to research laboratories and an extended care facility of 50 - 60 beds.

The hospital has an active surgical service with three residents, an oncology trainee and two full-time staff surgeons. In addition, there are a number of visiting surgeons in various surgical sub-specialties.

An active radiotherapy and radiology service is under the supervision of Dr. Ronald Messer and is affiliated with Boston University. Residents and junior staff personnel rotate to Pondville from the University Hospital and Boston City Hospital for radiotherapy and radiology training.

The Pathology Department is under the supervision of Dr. Lalita Gandbhir. The surgical and post-mortem material available affords an excellent opportunity for good clinico-pathological correlation through her staff and consultants. It has a modern autopsy room, surgical pathology laboratory and histology laboratory.

The Department of Medicine in the past was handled on a part-time basis by consulting internists. However, with the appointment of Dr. Leo Stolbach as Chief of Medicine on July 1, 1970, a full-time program for the medical service has been established with the following staff: a junior staff physician, a Tufts Oncology Fellow and two residents.

The patient population in 1972 represented 694 new patients. Since the hospital opened in 1927, a total of more than 55,000 patients have been cared for at Pondville. This affords an excellent opportunity for reviews of various malignant diseases. Hospital summaries are excellent and followup of patients is meticulous.

The medical service participates actively in protocol studies of the Eastern Cooperative Oncology Group (Principal investigator - Dr. Stolbach) and the Cooperative Breast Cancer Group (Principal investigator - Dr. Rita Kelley). This participation affords considerable experience to the house staff in gaining an understanding of carefully designed clinical research studies.

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Daily ward rounds and weekly grand rounds are made on all patients on the medical service. A combined Oncology Conference with the Lemuel Shattuck Hospital has been held on alternate Thursdays at the Pondville Hospital and on alternate Saturdays at the Lemuel Shattuck Hospital. The oncology staff as well as invited speakers conduct these conferences. Also, a weekly Tumor Board Conference is held at the Pondville Hospital to discuss all new patients admitted to the hospital. Members of the pathology, surgery, radiotherapy and medical departments attend this conference, which has been a valuable adjunct to the education of the residents and staff and has resulted in a better coordination of therapeutic efforts. It has been an excellent forum for exchange of ideas between the services at the hospital. Finally, a slide conference is held by the Pathology Department to review interesting patient material on a bi-weekly basis.

Dr. Charles Apffel is devoting full-time to basic research in the area of cancer immunology. His research interests have focused on the regulation of antigenic expression and the role of serum glycoproteins in tumor growth and regression. He has recently been involved in isolation of a protein on the surface of the tumor cell which apparently blocks the immunologic expression of the cell.

Dr. Stolbach's basic research interests have been mainly in the area of serum isoenzyme analysis in patients with malignancy. This work has been carried out in conjunction with the Tufts Cancer Research Laboratory under the directorship of Dr. William H. Fishman. In addition, his laboratory is presently involved in preliminary studies for the measurement of various tumor antigens. Plans are also underway to establish a tissue culture laboratory to grow tumor cells in order to facilitate the study of immunologic factors in host response of cancer patients.

Relationship of proposed research to total research effort of TCRC

The cancer cell phenotype program is at present utilizing ascitic fluids and tumor tissues of patients with ovarian cancer as source materials in the search for carcinoembryonic proteins which could be evaluated in cancer patients in general. We have reached the point where four of these (Regan isoenzyme, HCG, CEA and α-fetoprotein) merit systematic study.

Amongst types of human cancer, bronchogenic cancer would now become the focus of laboratory and clinical interest of the staff of the Center with respect to the relevance of carcinoembryonic proteins from all points of view.

If one thinks in terms of the overall effort, I believe that in the first year, this project could constitute 25% and the percentage of total effort would rise in successive years in proportion to the progress and decisive results obtained. Specifically, this project would establish our immunologic and radioimmunoassay capability in the Cancer Research Center laboratory by supporting Drs. Raam and Chang. (It would also give these young people a research opprtunity which they have merited.) Also, it would establish a productive branch in the Pondville Hospital and would bring to the Pathology Department the opportunity to be engaged in the research program of the Center. Both of these desiderata, if they can be managed, will meet two major handicaps to our progress in research.

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